Identification of Highly Potent and Selective Interleukin-1 Receptor-Associated Kinase 4 Inhibitors for the Treatment of Rheumatic Diseases

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ABSTRACT

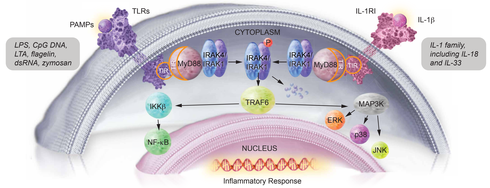
Background/Purpose: Interleukin-1 receptor-associated kinase 4 (IRAK4) is a key mediator of the innate immune response orchestrated by interleukin-1 receptor (IL-1R), interleukin-18 receptor (IL-18R), IL-33 receptor (IL-33R), and Toll-like receptors (TLRs). IRAK4 activation is mediated by MYD88, a common signaling adaptor protein downstream of these receptors. Mutations leading to inactivation or activation of MYD88 have been reported in patients with immune deficiencies and cancer, respectively. In addition, IRAK4-deficient humans are protected from chronic inflammatory diseases. Thus, IRAK4 is an attractive therapeutic target for the treatment of autoimmune diseases such as lupus. Historically, identification of potent and selective IRAK4 inhibitors has been challenging due to structural features in the catalytic binding site that block access to the hydrophobic back pocket. We have developed new structure-activity relationship (SAR) insights, including the identification of unstable (high-energy) hydration sites, which guide the design of potent and selective small molecule ligands.

Methods: Using this innovative structure-based approach, we designed, synthesized and tested small molecule inhibitors based on hits originating from a virtual screen. These novel compounds were profiled for IRAK4 kinase inhibition, selectivity, and drug-like properties. Furthermore, selected compounds were tested in THP1 cells, human peripheral blood mononuclear cells (hPBMCs) and whole blood for impact on LPS-, IL-1-, R848-, and/or CpG-mediated signaling. The inhibitors were also tested *in vivo* in acute LPS challenge, chronic collagen-induced arthritis (CIA), imiquimod-induced psoriasis and MSU air pouch gout models.

Results: Here, we feature three highly potent, selective IRAK4 inhibitors, ND-346, ND-2110 and ND-2158. The Kis of ND-346, ND-2110, and ND-2158 for IRAK4 are 50, 7.5 and 1 nM, respectively. These compounds are highly selective against 334 kinases, and are potent inhibitors of IL-1-induced IRAK1 degradation in MRC5 cells, LPS-, IL-1-, R848 (TLR-7 agonist)- and CpG (TLR-9 agonist)-induced cytokine production in hPBMCs and whole blood. Furthermore, these compounds are efficacious in all *in vivo* murine models tested, showing efficacy ≤30 mg/kg BID.

Conclusion: Utilizing unique and innovative structure-based drug design, we have rapidly discovered potent and selective IRAK4 inhibitors as potential drug candidates for the treatment of chronic rheumatic diseases.

IRAK4 Plays an Essential Role in Mediating IL-1/TLR Signaling to Pro-inflammatory Cytokine and Chemokine Production



Nimbus Approach Enables Rapid Discovery of Potent, Drug-like IRAK4 Inhibitors

Ideas	1.3 MM	3,000	60,000
Total compounds synthesized		40	<300
Potency	8,000 nM	100 nM	<10 nM
Milestone achieved			

Figure 1: Structure based modeling approaches were used to conduct a virtual screen of IRAK4 and led to the rapid optimization of lead matter. The technology allows the generation of a large number of "virtual compounds" (ideas) that are docked into the model from which the most promising were selected for synthesis. Time from screening to *in vivo* PoC was accelerated compared to typical drug discovery programs.

ND-2110 & ND-2158 Inhibit TNFα Production in Human PBMCs

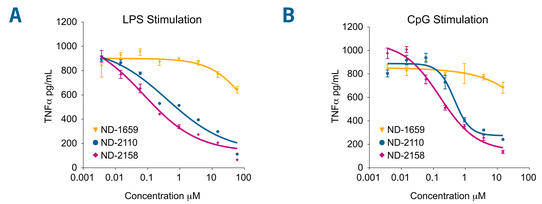


Figure 2: Human PBMC cultured serum free at a density of 100,000 cells per well (**A**) or 200,000 cells per well (**B**) were stimulated with 1 μg/mL LPS (A) for 5hrs; or stimulated with 0.5 μM CpG for 20 hrs (B). Cell supernatant was analyzed for TNFα production by ELISA. Negative control ND-1659 lacks IRAK4 inhibitory activity. IC_{50} values are reported in Table 1.

Potent and Selective IRAK4 Inhibitors Identified

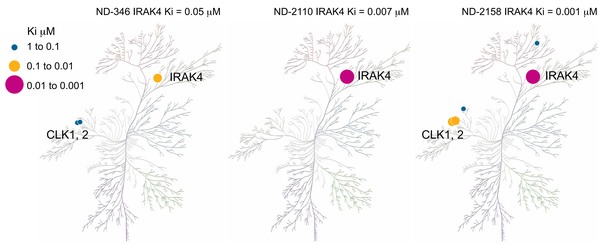


Figure 3: ND-2110 and ND-2158 are highly potent and selective IRAK4 inhibitors. Kinase inhibitory activity was evaluated using radioactive kinase assays at 10 μM ATP (Reaction Biology Corp.). Over 300 kinases were tested at 10 μM compound concentration and based on the % inhibition results, the kinases that were inhibited >50% were evaluated in IC_{50} dose response assays. Kinase Ki < 1 μM shown on kinome maps.

Table 1: Potency and Drug-Like Properties of IRAK4 Inhibitors

		ND-346	ND-2110	ND-2158
Biochemical IRAK4 Kinase Assay	IRAK4 Ki μM	0.05	0.007	0.001
Cytokine Production IC_{50} (μM)	THP1 cells (LPS -> TNFα)[1]	1.0	0.59	0.13
	PBMC (LPS-> TNFα)[2]	6.5	0.40	0.10
	PBMC (R848 -> TNFα)[3]	ND	0.67	0.09
	PBMC (CpG -> TNFα)[4]	ND	0.45	0.16
	Whole Blood (R848 -> TNFα)[5]	ND	0.54	0.77
Protein Binding[6]	Human PPB % Bound	63	73	82
Human in vitro Stability[6]	Cl_{int} (mL/min/kg) Microsomes	8	19	17
	Cl_{int} (mL/min/kg) Hepatocytes	7	21	12
Rat PK (IV) 3mpk[7]	Cl_{int} (ml/min/Kg)	113	67	72[8]
Rat PK (PO) 10 mpk[7]	$T_{1/2}$ (hr)	1.2	1.3	1.8[8]
	C_{max} (μM)	1.7	1.4	0.5[8]
	F (%)	94	25	11[8]
	AUC (μM hr)	1.4	1.5	1.5[8]
Physical properties	MW(Da), solubility (μM)	300 – 325, 830	400-425, 291	425-450, >300

[1]THP cells stimulated with 0.3 μg/mL LPS for 5 hrs; cell supernatant measured for TNFα production by ELISA; [2]See Figure 2A; [3]Human PBMC (100,000 cells per well) stimulated with 1 μM R848 for 5 hrs; cell supernatant measured for TNFα production by ELISA; [4]See Figure 2B; [5]See Figure 4; [6]Standard Assay performed at Pharmaron; [7]PK conducted in Sprague Dawley rat. IV formulation 10% HP-β-CD in saline; oral formulation 0.5% MC in saline; [8]PK conducted in female Lewis rat. Oral formulation ND-2158 (0.5% MC in saline)

ND-2110 & ND-2158 Inhibit TNFα Production in Human Whole Blood

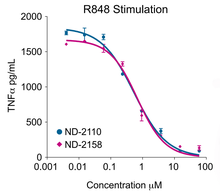


Figure 4: Human whole blood was diluted 1:1 with PBS and treated with IRAK4 inhibitors for 30 minutes prior to R848 stimulation at 1 μM. Plasma samples collected 24 hours post stimulation followed by TNFα ELISA analysis. IC_{50} values reported in Table 1.

ND-2110 Inhibits LPS-Induced TNFα Production in Rats

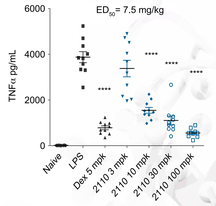


Figure 5: Female Lewis rats were administered drug PO 30 minutes prior to stimulating with LPS IV. Serum samples collected at 1 hr post LPS administration, followed by TNFα ELISA analysis. **** p < 0.001

IRAK4 Inhibitors Block IL-1β-induced IRAK1 Degradation in MRC5 Cells

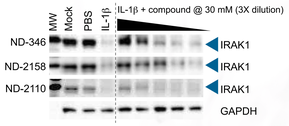


Figure 6: MRC5 cells were treated with IRAK4 inhibitor for 30 minutes prior to IL-1β stimulation for another 30 minutes. Cell lysates were subjected to western blot analysis for IRAK1, and GAPDH to confirm equivalent protein loading. Similar GAPDH signal was detected for all experiments, and representative is shown.

ND-2110 and ND-2158 Reduce Clinical Symptoms in Murine IMQ-Induced Psoriasis Model *in vivo*

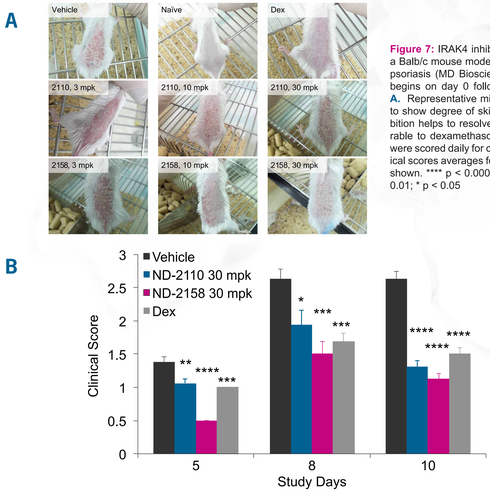


Figure 7: IRAK4 inhibitors are efficacious in a Balb/c mouse model of imiquimod-induced psoriasis (MD Biosciences). IP BID dosing begins on day 0 following IMQ application. **A.** Representative mice were photographed to show degree of skin scaling. IRAK4 inhibition helps to resolve psoriatic skin comparable to dexamethasone (Dex). **B.** Animals were scored daily for clinical symptoms. Clinical scores averages for days 5, 8, and 10 are shown. **** p < 0.0001; *** p < 0.001; ** p < 0.01; * p < 0.05

IRAK4 Inhibitors are Efficacious in MSU Air Pouch Model of Human Gout

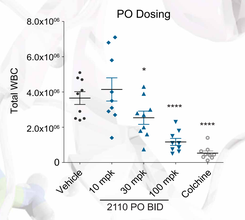


Figure 8: Balb/c mice were used to create a sterile air pouch, and mice were dosed for 6 days as indicated. Following last dose mice were anesthetized and the air pouch was injected with MSU suspension. Four hours later the air pouch exudate was collected and an aliquot is analyzed for cell counts. **** p < 0.0001; *** p < 0.001; ** p < 0.01; * p < 0.05

IRAK4 Inhibitors Block Collagen-Induced Arthritis in Mice

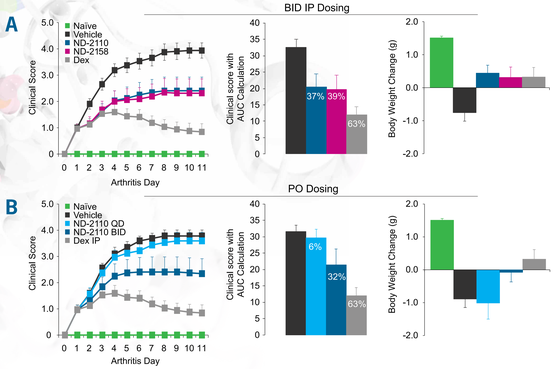


Figure 9: Male DBA mice, immunized with collagen on day 0 and 21, were randomly enrolled upon disease onset (n = 8 per group, except for n = 4 naïve controls). Day 1 is designated as the first treatment day, and mice were evaluated daily for clinical scores till day 11, shown as average scores on the left panels. Middle panels show the average clinical scores with AUC calculation, and % inhibition relative to vehicle is indicated in the bar graph. Mice were weighed on alternating days, and change in body weight on day 11 from day 1 is shown in the right panels. **A.** 2110 and 2158 were dosed at 30 mg/kg IP BID, and dexamethasone at 0.1 mg/kg IP BID, in vehicle 10% HPbCD. Left panel - IRAK4 inhibitors vs vehicle: p<0.05 days 2-11; dexamethasone (Dex) vs vehicle: p<0.05 day 1, p<0.01 day 2, p < 0.001 days 3-5, p <0.0001 days 6-11. Middle panel - IRAK4 inhibitors vs vehicle: p<0.5; dex vs vehicle p<0.0001. Right panel - 2158 and dex vs vehicle: p<0.05, 2110 vs vehicle IP BID p<0.01. **B.** 2110 was dosed at 30 mg/kg PO QD and BID, in vehicle 0.5% MC in saline. Left panel - 2110 BID vs vehicle: p<0.05 days 8-11. The control groups dexamethasone and naïve are the same as in A. All groups shown are part of the same study.

SUMMARY

- IRAK4 is a sought after target for the treatment of autoimmune diseases
- Previous attempts to identify small molecule modulators of IRAK4 resulted in poor selectivity and inadequate drug-like properties
- Using a physics-based computational approach, Nimbus has uncovered previously unexploited drivers of potency and selectivity. These insights were used to discover and design the first truly selective IRAK4 inhibitors
- Robust *in vivo* efficacy was subsequently demonstrated in collagen-induced arthritis, psoriasis and mono-sodium urate (MSU) gout mouse models
- Potent *in vitro* inhibition of cytokine production was observed in cells and whole blood
- Nimbus compounds have good drug-like properties and are candidates for further development for treating inflammatory diseases